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STUDY ON LONG-TERM EFFECTS OF NITRIC OXIDE* ON MICE
[Issankachisso no seitai ni oyobosu eikyo ni kansuru kenkyū]

by

Hajime Oda, M.D.*, Hiroshi Nyogami*, Shigeko Kusumoto*,
Etsuko Yonekawa*, Yasuko Tsubota, Taichi Nakajima, M.D.*,
Hideki Miyaji, M.D.** and Akihiko Kurata, M.D.**
* Division of Environmental Health Research, Osaka
Prefectural Institute of Public Health
** 2nd Department of Pathology, Hyōgo College of Medicine

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Foreword

Air pollution by nitrogen oxides is a phenomenon which has been progressing rapidly in recent years. Of particular concern are the cases of pollution by nitric oxide (NO) whose concentration is frequently

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* Translator's Note: "nitric oxide" was used to conform with English title given in the original but "Issankachisso" can also be translated as "nitric monoxide."

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observed to exceed the concentration of pollution by nitrogen dioxide (NO_2). As far as NO_2 is concerned, environmental standards have already been established on the basis of animal experiment data and epidemiological findings which revealed negative effects caused in the respiratory apparatus by low concentrations. In contrast, the establishment of environmental standard levels with respect to NO has been left neglected due to the absence so far of data regarding its effects at low concentrations.

In terms of animals exposed to high concentrations of NO, there have already been reports of such central nervous system disorders as spasms and paralysis as well as death due to methemoglobinemia [1], and of the formation of nitrosyl-hemoglobin (NOHb) due to NO combining with hemoglobin [2]. These findings indicate that the effects are general rather than restricted to the respiratory apparatus.

This report will present a general examination of the toxicity of NO, which we attempted by exposing mice for periods of up to 6.5 months to a concentration of 10 ppm, a level considerably lower than the several hundred ppm exposure concentrations previously reported and, at the same time, below the allowable concentration stipulated by the ACGIH for the industrial environment.

The tests we conducted consisted of the following items: determination of the body and vital organ weights; histopathological examination of the vital organs; hematological tests consisting of the determination of the red cell count, the hemoglobin level, the hematocrit value, the white cell count and the NOHb level; and biochemical blood tests consisting of the determination of the GPT and total bilirubin levels for studying the liver function, the determination of the urea nitrogen level for studying

the kidney function, the determination of the total cholesterol level in connection with fat metabolism, the determination of the level of cholinesterase, which is an SH enzyme, and the determination of the level of 2, 3-diphosphoglycerate (2, 3-DPG) which is known to have the function of regulating the oxygen affinity of hemoglobin.

Methodology

Animals: (ICR-JCL) incest-bred mice (♀) were subjected to exposure from the age of 4 weeks. After 2 weeks, 2 months, 4 months and 6.5 months respectively, a control group of 60 animals and an exposed group of 60 animals were removed for use in tests. With every 2 groups of 60 animals each, 15 animals of each group were used for the hematological tests, 15 of each for the biochemical tests, 10 animals of each for the NOHb level measurement and 10 animals of each for the determination of the vital organ weights and the histopathological examinations respectively.

NO Exposure: As described in our previous report [2], we added NO to ambient air filtered through a filter unit and activated charcoal, and then introduced the gas mixture into the animal exposure chambers after first removing the NO_2 formed in the mixture with soda lime. The NO concentration was at an average level of 10 ppm with a fluctuation range of approximately 10% while the concentration of NO_2 contamined [mixed] in was 1-1.5 ppm. The animals were exposed continuously day and night. We used a Saltzman measurement device (Horiba Model APNA-1S) for the determination of the NO and NO_2 concentrations. In addition, we monitored the NO concentration with a chemical luminescence type NO measurement device (Denki Kagaku Keiki Model GLN-21S) and used an electrical valve controlled by a PID

controller (Okura Denki Model EC-1322) built into the recorder for the automatic regulation of the gas flow volume. The control group mice were kept in chambers of the same type as-those of the exposed group, without the introduction of NO.

Body and Vital Organ Weights: The body weights were measured on the day before the start of exposure and, thereafter, approximately at 1 week intervals. The weights of the vital organs were determined by measuring the lungs, the liver, the spleen, the kidneys, the heart and the brain of mice killed by cervical dislocation and calculating their weight ratios with respect to the body weights.

Hematological Tests: Using blood samples collected from the orbital venous plexus, we determined the red cell counts and the white cell counts by means of a Tōa micro cell counter (Model CC-1002). The total hemoglobin levels and the hematocrit values were determined by the cyanmethemoglobin method and the micro-hematocrit method respectively. On the basis of the red cell counts, the total hemoglobin levels and the hematocrit values thus obtained, we calculated the mean red cell volumes (MCV), the mean red cell hemoglobin levels (MCH) and the mean red cell hemoglobin concentrations (MCHC). We also prepared blood smear samples and examined them under the microscope to obtain the white cell index (neutrocyte/lymphocyte ratio) values.

NOHb Level: As described in our previous report [2], we analyzed blood samples taken from the orbital venous plexus with an X-band electron spin resonance device (Nihon Denshi Model PE-3X) under the conditions of modulation width 6.3 gauss and temperature $\simeq$ 140°C, and then calculated the NOHb levels on a computer (Nihon Denshi Spectrocomputer

Model JEC-6) connected for on-line operation. It should also be noted that after every one of the above-designated time periods, the NOHb levels of a group of mice exposed for 1 hour were also recorded along with those of the control group.

Biochemical Blood Tests: Serum samples were prepared from blood collected from the orbital venous plexus, and their GPT levels were measured by a modified ~~Raltoman-Purankeru~~ Leitman-Frankel (GPT) method, the total cholesterol by the O-phthaldehyde method, the total bilirubin by the alkali azobilirubin bile method and the urea nitrogen level by the diacetylmono'oxim-thiosemicarbazide method. Also, whole blood samples with heparin added were used for determining the levels of 2, 3-DPG and cholinesterase by the enzymic method using a Calbiochem kit and by the DTNB method respectively.

Histopathological Examinations: The lung, liver, spleen, kidney, heart and brain samples used for the vital organ weight determination were fixed with formalin (some of the brain with Bouin's solution), and then packed in paraffin and stained with hematoxylin and eosin according to standard procedures. In addition, argentaffine fiber staining, PAS, PT, AH and fat (Sudan III) stainings were also carried out as needed. In the case of the brain samples, Nissl staining and LFB staining were combined for microscopic examination.

Results

When the numbers of dead mice in the 6.5 month groups were totaled, it was found that there was no significant difference between the control group and the exposed group which had respectively 3 dead animals out of 60 and 4 animals out of 60. Among the mice of the exposed group, loss

of hair and the yellowing of body hair were observed together with increased slowness of movement. No difference could be observed between the two groups in terms of body weight throughout the 6.5 month period (Table 1)

As Table 1 shows, there were no differences between the groups in the ratios of the vital organ weights to the body weight in terms of the liver, the heart and the brain. However, there was a significant increase in the kidney weight for the exposed groups after 2 weeks, while the lung and the spleen exhibited tendencies to increase in weight after each of the time periods with significant differences in the cases of 2 weeks and 6.5 months.

The fluctuations in the levels of blood componenets are shown in Table 2. A significant increase in the white cell count was observed in the exposed groups after 2 weeks, and trends toward increased count continued to be observed thereafter. In terms of the leuco-white cell index, a higher value was seen in the group exposed for 2 months. In addition, there was a greater variance of the values in the group exposed for 6.5 months compared to the control group with some individual values showing an increase. No significant differences, however, could be observed between the groups throughout the entire exposure period in terms of the red cell count, the total hemoglobin level, the hematocrit value, and the red cell indices of MCV, MCH and MCHC.

The levels of NOHb, which was not detected in the control group, were 0.23% for the 6.5 month group and 0.23% again for the 1-hour exposure group. Similarly, no difference could be found in the NOHb levels between

the long-term exposure group and the 1-hour exposure group in the cases of 2 weeks, 2 months or 4 months.

In the biochemical blood test values,-no significant differences could be observed between the exposed groups and the control groups throughout the entire exposure period in terms of the GPT, total cholesterol and the urea nitrogen levels of the serum samples as indicated in Table 3. The total bilirubin levels exhibited a tendency to be higher in the exposed groups after 2 weeks to 4 months. With cholinesterase, increases in the activity values due to aging were detected in both groups, but after 2 months, it was found that the activity value for the exposed groups had become lower compared to the control groups. No significant differences could be observed in the 2, 3-DPG levels with the exception of a decrease in the 4-month exposure group.

Key to Table 1 on Page 8:

1. weeks
2. months
3. body weight
4. control group
5. exposed group
6. lung
7. liver
8. spleen
9. kidney
10. heart
11. brain

Key to Table 2 on Page 9:

1. weeks
2. months
3. white cell count
4. control group
5. exposed group
6. leuco-white cell index
7. red cell count
8. total hemoglobin level
9. hematocrit value

Table 1. Body weights and vital organ weights (body weight ratios)

		2 1週	2 2カ月	4 2カ月	6.5 2カ月
3 体重 (g)	対照群	24.3 ± 1.8	30.9 ± 2.7	33.8 ± 3.2	34.4 ± 3.5
	暴露群	24.7 ± 1.4	31.1 ± 2.1	33.9 ± 3.0	34.0 ± 3.3
6 肺重量 (%)	対照群	0.60 ± 0.04	0.60 ± 0.06	0.56 ± 0.06	0.59 ± 0.05
	暴露群	0.64 ± 0.03*	0.63 ± 0.06	0.62 ± 0.08	0.65 ± 0.04**
7 肝重量 (%)	対照群	5.39 ± 0.46	5.17 ± 0.55	4.62 ± 0.49	4.66 ± 0.32
	暴露群	5.48 ± 0.34	4.84 ± 0.38	4.57 ± 0.17	4.57 ± 0.54
8 腎重量 (%)	対照群	0.34 ± 0.09	0.43 ± 0.05	0.35 ± 0.08	0.34 ± 0.06
	暴露群	0.59 ± 0.12**	0.50 ± 0.13	0.38 ± 0.05	0.45 ± 0.11*
9 脾重量 (%)	対照群	1.47 ± 0.08	1.39 ± 0.23	1.38 ± 0.18	1.35 ± 0.12
	暴露群	1.56 ± 0.04**	1.43 ± 0.17	1.38 ± 0.11	1.37 ± 0.13
10 心重量 (%)	対照群	0.46 ± 0.03	0.48 ± 0.07	0.43 ± 0.04	0.44 ± 0.04
	暴露群	0.47 ± 0.02	0.44 ± 0.04	0.45 ± 0.07	0.46 ± 0.04
11 脳 (%)	対照群	1.92 ± 0.15	1.71 ± 0.12	1.61 ± 0.10	1.68 ± 0.11
	暴露群	1.99 ± 0.14	1.67 ± 0.18	1.62 ± 0.09	1.64 ± 0.13

*P < 0.05 **P < 0.01

Table 2. Hematological test values

		2週	2カ月	4カ月	6.5カ月
白血球数 (×10³/mm³)	対照群	5.56 ± 1.10	5.73 ± 0.96	6.16 ± 1.90	4.67 ± 1.22
	暴露群	8.04 ± 1.67**	5.82 ± 1.10	7.51 ± 2.12	5.59 ± 1.76
白血球指数	対照群		0.14 ± 0.06	0.22 ± 0.13	0.33 ± 0.09
	暴露群		0.20 ± 0.05**	0.26 ± 0.13	0.35 ± 0.17
赤血球数 (×10⁴/mm³)	対照群	853 ± 52	840 ± 49	831 ± 26	840 ± 52
	暴露群	833 ± 66	834 ± 34	862 ± 58	840 ± 45
総ヘモグロビン量 (g/dl)	対照群	14.4 ± 0.6	15.5 ± 0.7	14.4 ± 0.7	14.7 ± 0.8
	暴露群	14.4 ± 1.0	15.7 ± 0.5	14.8 ± 0.7	14.5 ± 0.9
ヘマトクリット値 (%)	対照群	48.1 ± 1.8	47.3 ± 2.0	46.1 ± 2.2	45.8 ± 2.1
	暴露群	48.7 ± 2.5	48.2 ± 1.7	47.8 ± 2.4	45.3 ± 2.7
MCV (μ³)	対照群	57.0 ± 3.0	55.9 ± 2.2	56.0 ± 2.5	55.3 ± 3.8
	暴露群	58.6 ± 3.5	57.8 ± 1.8*	55.5 ± 2.2	54.3 ± 2.8
MCH (μμg)	対照群	17.1 ± 0.8	18.5 ± 1.0	17.5 ± 0.7	17.5 ± 1.0
	暴露群	17.4 ± 1.2	19.0 ± 0.6	17.2 ± 0.9	17.4 ± 0.9
MCHC (%)	対照群	30.0 ± 0.6	32.9 ± 0.7	31.2 ± 0.5	32.1 ± 0.6
	暴露群	29.8 ± 0.7	32.3 ± 0.9	30.9 ± 0.8	31.9 ± 0.5

*P < 0.05　　**P < 0.01

Table 3. Biochemical test values

		2 週	2 カ月	4 カ月	6.5 カ月
GPT (単位)	対照群	53 ± 19	24 ± 8	21 ± 6	27 ± 10
	暴露群	38 ± 15	25 ± 7	27 ± 11	26 ± 6
総コレステロール (mg/dl)	対照群	154 ± 22	123 ± 17	136 ± 24	117 ± 11
	暴露群	166 ± 31	124 ± 23	127 ± 32	109 ± 16
総ビリルビン (mg/dl)	対照群	0.22 ± 0.09	0.24 ± 0.09	0.28 ± 0.08	0.34 ± 0.12
	暴露群	0.27 ± 0.05	0.36 ± 0.07**	0.34 ± 0.07	0.34 ± 0.12
尿素窒素 (mg/dl)	対照群	40.4 ± 7.5	39.3 ± 4.9	27.9 ± 3.8	29.9 ± 3.9
	暴露群	40.3 ± 7.0	36.0 ± 5.0	27.0 ± 5.1	27.6 ± 4.6
コリンエステラーゼ (μmol/分/ml全血)	対照群	4.01 ± 0.38	7.36 ± 0.96	9.09 ± 2.20	9.55 ± 1.71
	暴露群	4.03 ± 0.55	6.83 ± 1.14	7.41 ± 0.74*	7.89 ± 1.25*
2, 3 −DPG (μmol/ml RBC)	対照群	8.46 ± 0.38	8.42 ± 0.34	8.09 ± 0.31	8.78 ± 0.45
	暴露群	8.17 ± 0.47	8.53 ± 0.37	7.85 ± 0.24*	8.76 ± 0.49

*P < 0.05 **P < 0.01

Key to Table 3 on Page 10:

1. weeks
2. months
3. (units)
4. control group
5. exposed group
6. total cholesterol
7. total bilirubin
8. urea nitrogen
9. cholinesterase
10. (umol/min/ml whole blood)

The macroscopic observation of the extracted organs indicated, first of all, that the lungs of the 2 month, 4 month and 6.5 month exposure groups were generally harder and stronger in the dark red coloration of the sections compared to the lungs of the control groups, suggesting some type of pathological change. The basic histopathological changes seen in the lung tissues (Photos 1-4) consisted of the degeneration and necrosis of the mucous membrane together with a subsequent proliferative reaction, and hypertrophic enlargement of the alveolar septa due to hyperemia and congestion. The changes in the bronchi consisted of the degeneration and necrosis of the mucous epithelia during the acute period, but proliferative reaction became more prominent particularly in the bronchioli as the condition became chronic, our principal observations being papillary proliferations, goblet cell formations and images ot bronchial obstruction by mucous substance. A slight infiltration of round cells could be observed in the submucous layer but no change could be detected in the basilar membrane or the smooth muscle layer. It may be assumed that the above changes become prominent with passage into the chronic period and apparently become established in mice exposed for 4 months. The changes in the alveolar septa were observed throughout the acute and chronic periods with the conditions ranging from localized changes to serious changes affecting

entire lobes. The inflammatory changes accompanying this condition were relatively minor, which is to say only to the extent of an occassional round cell infiltration being observed. As far as the heart, the liver, the kidneys, the spleen and the brain are concerned, we could not detect any indications to suggest a significant difference between the exposed groups and the control groups. In particular, we did not observe any changes indicating the occurrence of hypoxemia.

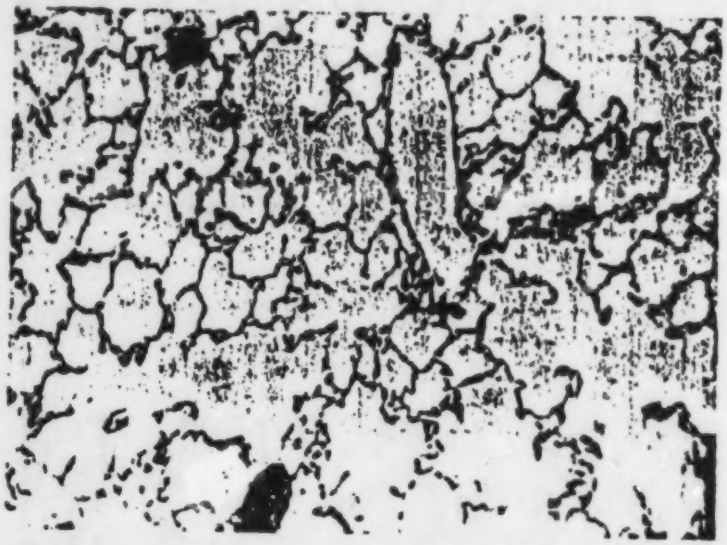

Photo 1. Control group lung tissue (6.5 months)

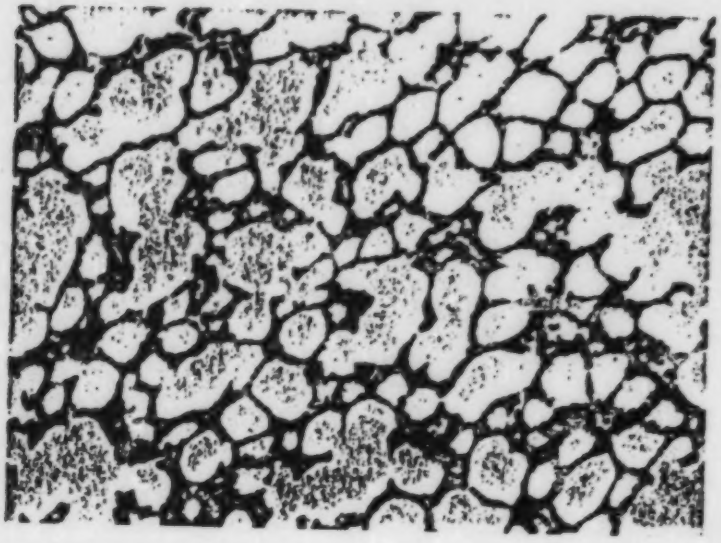

Photo 2. Exposed group lung tissue (6.5 months)

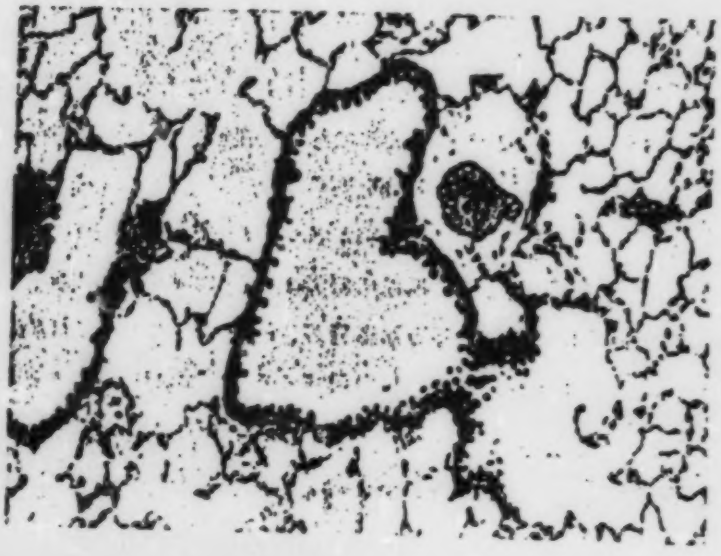

Photo 3. Control group bronchiolar tissue (6.5 months)

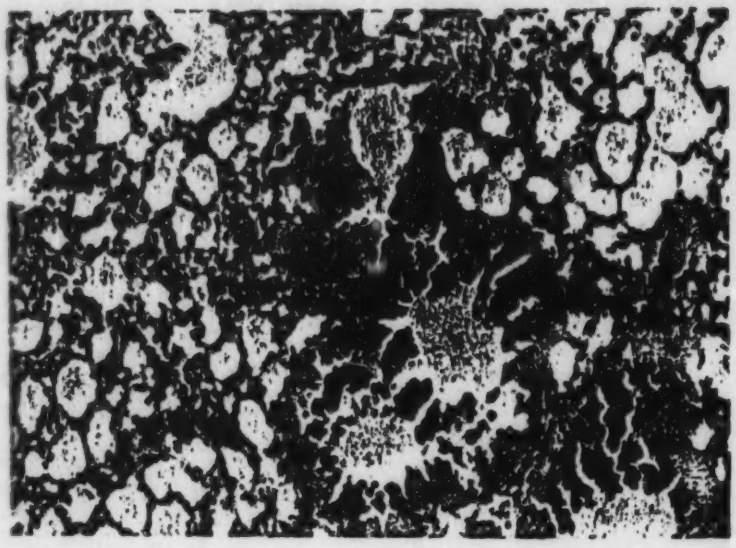

Photo 4. Exposed group bronchiolar tissue (6.5 months)

Discussion

Since the regions of living bodies which come into contact with air pollutants are generally restricted to the respiratory apparatus, the effects of the pollutants are usually considered in terms of 2 categories; namely, the effects on the respiratory apparatus and the effects on other regions (general systems). For example, the effects of sulfur oxides and NO_2 are mostly observed in the respiratory apparatus while general effects are usually considered in the case of carbon monoxide. The affected regions thus vary according to the differences in the physiochemical properties of the pollutants, and one may assume that substances whose capture rate by the respiratory apparatus is high would affect the respiratory apparatus while substances whose capture rate there is low would enter the bloodstream from the lungs in greater quantities to produce general effects. However, it has been learned through progress in research that carbon monoxide, which has been assumed

to have only minor effects on the lung tissues, does in fact cause damage to the lungs. There has also been a report that enzymic activities in the blood are affected by NO_2 infiltrating the bloodstream or they are secondary effects accompanying lung damage. In studying the biological effects of air pollutants, therefore, it is necessary to examine the general effects as well as to place importance on their effects on the respiratory apparatus, the main area of contact.

In Table 4, we compiled various published reports, including our own, which are apparently relevant to the evaluation of animal exposure experiment data on NO and of the toxicity of NO. Pflesser detected a higher methemoglobin level and central nervous system symptoms in mice he exposed to NO [1]., while Oda et al. confirmed the formation of NOHb in the blood of animals exposed to NO and suggested the possibility of NO infiltrating the bloodstream to cause general effects [2]. Meanwhile, Lewis et al. exposed beagles to low concentration NO over a long period of time and detected changes in their respiratory function [3]. However, it is still to early to say that an adequate amount of research data is available concerning the toxicity of NO. Even with regard to the individual findings, the dose-response relations are too unclear for any conclusive evaluation of the toxicity of low concentration NO to be drawn at this time.

As for the reason behind the dearth of findings on the toxicity of NO, one should cite the fact that its toxicity has been largely neglected until the recent years because of its low pollution level and its lack of mucosal irritability. In addition, NO exposure tests were considered difficult to conduct due to the mixed presence of NO_2. Having succeeded

in removing NO_2 to a relatively satisfactory extent by means of soda lime after a review of the exposure methods [2], we carried out on this occasion an exposure test of up to 6.5 months at 10 ppm as a first step in our investigation of the chronic effects of NO.

While it is known that a condition of oxygen deficiency due to CO exposure results in a compensatory increase in the amount of red cells, no notable fluctuation could be detected in the red cell system during our experiement. In addition, there were no histopathological indications in the brain or the heart to suggest hypoxemia, nor was there any sign of hypertrophy of the heart. On the basis of these observations, it is assumed that an oxygen-deficient condition of the tissues was not induced under the exposure conditions of our present experiements.

We reported earlier that the blood NOHb level exhibits a dose-response relation with the NO concentration of the exposure and that the level becomes constant after an exposure of about 20 minutes [2]. While the formation of an average level of 0.23% of NOHb was observed after a 6.5-month exposure to NO, the same level was also exhibited by 1-hour exposure groups as well as by 1-hour exposure groups which were exposed together with the 2-week, 2 months and the 4-month exposure groups. These findings confirmed that the NOHb level does not rise above a fixed concentration even after long sustained periods of exposure.

Table 4. Effects of NO

1作用	2報告者(年次)	3対象	4濃度・時間	5備考	6文献
7呼吸器	Murphy 5ら (1964)	9モルモット	16～50 ppm 10 4時間	11一分換気量、呼吸数に変化なし	4
	Shiel 12 (1967)	12イヌ	0.5～2% 13 7～50分	14肺水腫、出血、気管支肺炎、気管支上皮の増生	5
	15大道ら (1974)	16モルモット 線毛上皮	50 ppm 17 4分	15線毛運動能が半減 (in vitro)	6
	Lewis 9ら (1974)	19ビーグル犬	1.6 ppm 20 5ヶ月	21気気量の増加	3
	Kagawa 9ら (1975)	22小学生	23小学校(東京)での6ヶ月間	24最大呼気流量は環境中NO濃度に逆相関	7
NOHb	Toothill (1967)	25イヌ	2% 26 4～50分	27Met Hb 増加、NOHb は分光的に検出せず	8
	Sancier 9ら (1965)	29ラット	10ppm 27 1, 日	30NOHbはESRで検出せず	9
	Oda 9ら (1975)	31マウス ラット	10 ppm 32 1時間	33NOHbをESRで検出、20分で飽和し、量は0.13%	2
34死亡 その他	Flury (1930)	35最重性暴露者		36Met Hb 増加、中枢神経系症状を伴うNO_2中毒はNOによるであろう	10
	Pflesser (1930)	37マウス	38 310 ppm 以上 1時間以内	39Met Hb 増加、中枢神経系症状、死亡、NOはNO_2の4倍有毒	1
	Krasna 9ら (1954)	Proteus vulgaris	20～10,000 ppm	40Hydrogenase 活性低下	11
	Gray (1959)			41NO_2はNOの数倍有毒	12
	Greenraum 9ら (1967)	42イヌ	0.5～2% 43 7～50分	44Met Hb 増加、血液酸素分圧、pH低下、肺水腫で死亡	13

Key to Table 4 on preceeding page:

1. Effect published year
2. Author (~~in chronological order~~)
3. Subject
4. Concentration, time
5. Remarks
6. Bibliographical reference
7. Respiratory apparatus
8. et al.
9. Guinea pigs
10. 4 hours tidal
11. No change in ~~per minute ventilation~~ volume or respiratory frequency
12. Dogs
13. 7-50 minutes
14. Pulmonary edema, hemmorhage, bronchopneumonia, hyperplasia of bronchial epithelia
15. Ōmichi et al.
16. Ciliated epithelia of guinea pigs
17. 14 minutes
18. One-half reduction of ciliary movement (in vitro)
19. Beagles
20. 51 months
21. Increased ~~respiratory reserve~~ residual volume
22. Elementary school students
23. 6 months at an elementary school (in Tokyo)
24. Inverse correlation between maximum ~~exhaled air volume~~ and environmental NO concentration expiratory flow rate
25. Dogs
26. 4-50 minutes
27. MetHb level higher, NOHb not detected spectroscopically
28. Rats ?
29. 1 day, ~~10~~ days
30. NOHb not detected by ESR
31. Mice, rats
32. 1 hour
33. NOHb was detected by ESR and found to reach saturation in 20 minutes at 0.13%
34. Death, other
35. Occupationally exposed subject[s]
36. MetHb level higher, central nervous system symptoms accompanying NO_2 poisoning probably due to NO
37. Mice
38. Over 310 ppm
39. MetHb level higher, central nervous system symptoms, death, NO 4 times as toxic as NO_2
40. Reduced hydrogenase activity
41. NO_2 several times as toxic as NO
42. Dogs

(contd.)

Key to Table 4 continued)

43. 7-50 minutes
44. MetHb level higher, reduced blood oxygen partial pressure, lower pH level, death due to pulmonary edema

Among the weight ratios of the vital organs to the body weight, we noted that the lung weight either increased or tended to increase. This change was assumed to be attributable to a tendency toward a higher white cell count and to a change in the ~~white cell~~ leuco- index (more neutrocytes) as well as to the histopathological changes observed in the lung ~~samples~~ tissues. These histopathological changes in the lungs, namely hyperplasia of the bronchial mucous membrane and hypertophic enlargement of the alveolar septa, are similar to the already established changes known to be caused in the lungs by NO_2. Thus the changes in question may conceivably be attributed to the effects of NO_2 mixed in the NO used for the exposure, but they may just as well be due to NO itself. It would therefore seem necessary to conduct an NO exposure test with even lower concentration at which the mixed presence of NO_2 can be ignored.

The fact that either increases or tendency toward increase were observed in the weight ratios of the spleen to the body weight would suggest the occurrence of biological reactions in the hematocytic systems and the lymphatic immune system. This is an interesting question from the standpoint of reactions of the biophylactic mechanism, and should be pursued in greater detail in future studies.

Among the results of the biochemical blood tests, we noted the reduced activity of cholinesterase after exposures of 2 months to 6.5 months, which suggested inhibitive action of NO on the SH radical, cholinesterase being an SH enzyme. If such reduced cholinesterase

activity could also occur in the nervous system, it would also account for the possible effect of NO on the central nervous system. Also, if the decompositon of acetylcholine in the respiratory tract could be inhibited by reduced cholinesterase activity, it could conceivably result in enhanced sensitivity of the respiratory tract.

As described so far, various changes were detected following long term exposures to 10 ppm of NO. But before arriving at a conclusion on whether these changes are due to NO alone or they are also related to the mixed presence of NO_2, it is believed necessary not only to investigate the chronic effects of NO by lowering its concentration to the point of being able to ignore the mixed NO_2, but also to determine the causes behind the various changes observed in the current test.

In comparing NO and NO_2, the only nitrogen oxides belived to affect living bodies, one would note that NO has lower solubility in water than NO_2 and that NO does not possess the oxidative property of NO_2. From these properties of NO, one would anticipate that its capture rate by the lungs is lower than that of NO_2 and that NO by itself would not exhibit the "irritability" of NO_2. Wagner has reported that most of NO and NO_2 inhaled is retained in the system without being exhaled [14]. But nothing is yet known about such details as the rates of the gases captured in the lung tissues or their rates transferred to the blood-stream. Although the rate of invasion of foreign matter into the system or its distribution in the organs is routinely determined with the use of radioactive isotopes, such a method cannot be applied to nitrogen oxides due to the inavailability of radioactive isotopes of

either N or O of a long half-life. One would therefore have to resort to the use of the stable isotope N^{15} and conduct the measurement by mass spectrometry or spectroscopy. The investigation of internal distribution, which is generally important in connection with research into the toxic properites of environmental pollutants, should be implemented as soon as possible especially with respect to research on the nitrogen oxides. At the same time, since NO forms NO_2 through oxidation and the latter reacts with water to give nitrite ions and nitrate ions, it would seem necessary to take these products into consideration as well in the determination of the toxicity of NO.

Summary

ICR-JCL mice (♀) were exposed to 10 ppm of NO (1-1.5 ppm of NO_2 contaminated ~~mixed in~~) for periods of up to 6.5 months. After 2 weeks, 2 months, 4 months and 6.5 months respectively, 60 animals of the exposed group and 60 animals of the control group were used for the determination of the body weights, the vital organ weights, the white cell count, the leuco-white cell index, the red cell count, the total hemoglobin level, the hematocrit value, the NOHb level, GPT, total cholesterol, total bilirubin, urea nitrogen, 2, 3-DPG and cholinesterase and for histopathological examination of the various vital organs.

In the exposed animals, increased lung and spleen weights, higher white cell count, higher total bilirubin value and reduced cholinesterase activity were observed along with degeneration and necrosis of the pulmonary and bronchial mucous membranes and subsequent hyperplasia of

of the epithelial cells and hypertrophic enlargement of the alveolar septa due to hyperemia and congestion.

It was not possible to conclude that these changes were due to NO alone solely on the basis of these tests because of the possibility of the participation of the mixed NO_2. We therefore intend to expose animals to even lower concentrations of NO in the future in order to reduce the amount of NO_2 mixed in so that we may determine the dose-response relations involved in these changes.

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